Attachment H

Proposal # 2001- K204 (Office Use Only)

PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Using Molecular Techniques to Preserve Genetic Integrity of Endangered Salmon 'ing Supplementation Program

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Amount offunding requested $400,000

Some entities charge different costs dependent on the source of the funds. If it is different for state or federal funds list below.

Statecost $356,190 Federal cost *$400,000*

Cost share partners? ___Yes X No

Identify partners and amount contributed by each

Indicate the Topic for which you **are applying (check only one box).**

- ☐ Natural Flow Regimes
- ☐ Nonnative Invasive Species
- ☐ Channel Dynamics/Sediment Transport
- ☐ Flood Management
- ☐ Shallow Water Tidal/ Marsh Habitat
- ☐ Contaminants
- ☐ Beyond the Riparian Corridor
- ☐ Local Watershed Stewardship
- ☐ Environmental Education
- ☐ Special Stabs Species Surveys and Studies
- ☒ Fishery Monitoring, Assessment and Research
- ☐ Fish Screens

What county or counties is the project located in? Sonoma

What CALFED ecozone is the project located in? See attached list and indicate number. Be as specific as possible Bodega Marine Lab using samples from ecozones 3.1, 3.2, *4.4*

Indicate the type of applicant (check only one box):

- ☐ State agency
- ☐ Public/Non-profit joint venture
- ☐ Local government/district
- ☒ University
- ☐ Other:
- ☐ Federal agency
- ☐ Non-profit
- ☐ Tribes
- ☐ Private party

Indicate the primary species which the proposal addresses (check all that apply):

- ☐ San Joaquin and East-side Delta tributaries fall-run chinook salmon
- ☒ Winter-run chinook salmon
- ☐ Spring-run chinook salmon
- ☐ Late-fall run chinook salmon
- ☐ Fall-run chinook salmon
- ☐ Delta smelt
- ☐ Longfin smelt
- ☐ splittail
- ☐ Steelhead trout
- ☐ Green sturgeon
- ☐ Striped bass
- ☐ White Sturgeon
- ☐ All chinook species
- ☐ Waterfowl and Shorebirds
- ☐ All anadromous salmonids
- ☐ Migratory birds
- ☐ American shad
- ☐ Other listed T/E species: _____

Indicate the type of project (check only one box):

- ☒ Research/Monitoring
- ☐ Watershed Planning
- ☐ Pilot/Demo Project
- ☐ Education
- ☐ Full-scale Implementation

Is this a next-phase of an ongoing project? Yes ✓ No___
Have you received funding *from* CALFED before? Yes ✓ No___

If yes, list project title and CALFED number DWR 97-0330-B-81182 Spring Run Chinook

Have you received funding from CVPIA before? Yes ✓ No___

If yes, list CVPIA program providing funding, project title and CVPIA number (if applicable):

US FWS Coop. #1448-11-330-97-J194 Winter Run Chinook

By signing below, the applicant declares the following:

- The truthfulness of all representations in their proposal;
- The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and
- The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

DENNIS HEDGECOCK
Printed name of applicant

Dennis Hedgcock
Signature of applicant

Sandra M. Dowdy
Contracts and Grants Analyst
Printed name of authorized signature

Sandra M Dowdy
Authorized signature

Project Title: Using Molecular Techniques to Preserve Genetic Integrity of Endangered Salmon in a Supplementation Program

Amount Requested: $400,000 over 2 years (2001/2, 2002/3)

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Executive Summary

The success of supplementation programs for endangered species is crucially dependent upon the maintenance of genetic variation and enhancement of effective population size. This is especially true when the size of the natural population has fallen precipitously low, such as in the case of California's winter-run chinook salmon (*Oncorhynchus tshawytscha*). Few hatcheries have integrated molecular genetics with an artificial rearing program. However, since 1998, we have used molecular markers developed at the Bodega Marine Laboratory (UC Davis) to aid in selecting winter-run broodstock for a supplementation program at Livingston Stone National Fish Hatchery. We have developed a rapid procedure for identifying winter-run individuals, trapped at the barrier dam on the main stem of the Sacramento River during broodstock harvest, to ensure that they are not inadvertently hybridized with conspecific runs. In addition, by using microsatellite markers to identify the families of returning spawners, we can document the population dynamics of hatchery-spawned salmon and assess directly their genetic impact on the naturally spawning population. We propose to use molecular and population genetic techniques to evaluate hypotheses relating to the following objectives: 1) identify winter-run individuals prior to artificial propagation, 2) develop new polymorphic molecular markers for use in winter-run pedigree and linkage disequilibrium analyses, 3) genotype returning adult carcasses to obtain more precise winter-run size estimates, 4) genotype outmigrating juveniles to refine run-size estimates. 5) assess the naturally spawning population of winter run in Battle Creek, and 6) investigate further, by verifying models of effective population size, the impact of the supplementation program on the naturally spawning winter run. Our research supports the CALFED goal of restoration and recovery of an "at-risk species" by implementing techniques to preserve genetic integrity of winter-run chinook salmon and assess the effect of supplementation on the recovery of this endangered population.

Project Description

1. Statement of the Problem

Problem

Although artificial propagation programs can play a key role in the conservation of endangered species. there are a number of potential pitfalls associated with these approaches. These include hybridization between genetically distinct populations and over production of a few genotypes that swamp the wild population, resulting in a loss of genetic variation. Hatcheries are used to enhance natural stocks through artificial propagation of eggs and juvenile stages for release into the wild. Large-scale supplementation programs are already in place for numerous anadromous fish species. Nevertheless. these programs pose risks to the genetic integrity of imperiled populations, because the release of offspring reared in hatcheries from a relatively small number of adults can lead to swamping of natural genetic diversity. In situations where multiple stocks overlap both temporally and spatially. another concern is inadvertent hybridization between genetically distinct populations. These risks pose major challenges to ecosystem restoration as genetic diversity cannot be regained once lost. This project explores the use of molecular genetic techniques to increase the effectiveness of hatchery programs by ensuring that these problems are avoided.

Having plummeted from annual runs of nearly 100.000 fish in the late 1960s to less than 200 fish in 1991 and 1994, winter-run chinook salmon (*Onchorynchus tshawytscha*) was protected under both state and federal endangered species laws in the early 1990s. A hatchery supplementation program was initiated by the U.S. Fish and Wildlife Service (USFWS) under permits from the National Marine Fisheries Service (NMFS) and the California Department of Fish and Game (CDFG). Each year. USFWS captures up to 15% of the spawning run or 120 adults (whichever is larger) from the Sacramento River for maturation and spawning. Genetic research in support of the USFWS's winter-run chinook captive propagation and captive broodstock program takes place at UC Davis' Bodega Marine Laboratory (BML). **A** separate Central Valley chinook stock discrimination project resulted in the development of a set of DNA markers able to discriminate winter-run chinook from other runs of salmon (Banks et al. 2000). Using these techniques, we can assist in maintaining the genetic integrity of the endangered winter-run by preventing hybridization with other runs and evaluate the impact of hatcheries on the naturally spawning population.

Our research objectives are to 1) identify individual salmon adults for use in USFWS's winter-run chinook salmon captive propagation and broodstock programs, 2) develop new polymorphic markers to increase the power of pedigree analysis, **3)** genotype and identify to run origin (e.g., winter/non-winter) salmon carcasses collected in the mainstem Sacramento River and Battle Creek carcass surveys for population assessments and effective population size (N_e) validation, **4)** genotype and identify to run origin juveniles collected from rotary screw trap operations at the Red Bluff Diversion Dam and Battle Creek for population assessments and N_e validation, 5) assess the naturally spawning population of winter run in Battle Creek, and ***6)*** determine genetic impacts of the Supplementation program on the naturally spawning population through genetic analysis and verification of an effective population size (N_e) model.

Conceptual Model

The impact of hatchery supplementation on genetic diversity is mediated through effects on the effective size (N_e) of the natural population. N_e determines the rate at which deleterious mutations are fixed through the process of random genetic drift, reducing fitness and increasing chances of population extinction. N_e is a theoretical construct, the size of a mathematically ideal population that has rates of genetic drift and inbreeding equivalent to those in an actual population under study. In the mathematically ideal population, there are equal numbers of both sexes, adults mate at random, and variance in number of offspring per adult is binomial or Poisson. The number of adults N in the ideal population is, by definition, equal to the effective size, and the ratio of $N_e/N = 1.0$ in the ideal case. In actual populations, the sexes may not be in equal numbers, mating may not be at random. or the variance in offspring number may be larger than binomial or Poisson. Consequently, the N_e/N ratio for most vertebrate populations is thought to lie between 0.25 and 0.75 (Nunney 1992).

Conservation biologists have discussed a number of minimum effective population numbers. From the standpoint of protecting against inbreeding depression, which increases at a rate of $1/2N_e$ per generation. effective sizes of SO and above would appear to be sufficient (Franklin 1980). However. to avoid long-term loss of variation or to conserve rare alleles that might be the basis of future adaptation. effective sizes above a minimum 500 or even 5000 may be needed (Franklin 1980; Lande and Barrowclough 1987). Traditionally, N_e has been difficult to estimate for natural populations, although a variety of methods has emerged in the past decade (Waples 1991; Pudovkin et al. 1996; Luikart and Cornuet 1999). For a hatchery-supplemented population, N_e depends on the effective sizes of the hatchery and wild components of the population and on the relative proportion of hatchery origin fish (Ryman and Laikre 1991):

$$N_e = \frac{N_{eh} \times N_{ew}}{x^2 N_{ew} + y^2 N_{eh}}$$

N_{eh} and N_{ew} are the effective sizes of the hatchery and wild components of the population. respectively, and **x** and y are their relative contributions to the total ($x + y = 1.0$). In this model, only three independent parameters are necessary to describe the impact of hatchery enhancement .on natural biodiversity. Thus, a simple theory is available for evaluating the genetic impact of hatchery supplementation, reducing uncertainty of hatchery operation and providing the baseline data necessary to guide annual management decisions. The cumulative high-quality, long-term database this project provides (in tandem with other concurrent genetic stock assessments) will underpin the ecosystem-wide assessment of Central Valley chinook salmon. Without these data it is impossible to assess population integrity either biologically or legally under the ESA. Only through the use of molecular genetic analysis can hatchery intervention support the preservation and enhancement of wild stocks. This model links assessment and intervention as a preliminary step for future hatchery practice.

Hypotheses Being Tested

Using molecular markers to identify winter-run chinook is critical for the effective management of endangered salmon populations. The molecular techniques we have developed enable us to conduct rapid response genetic analysis on returning spawners to determine their run origin before they are transported to the hatchery. This procedure is vital to prevent inadvertent hybridization between genetically distinct runs, an error that occurred prior to 1998 before the inclusion of generic factors in broodstock selection. It is imperative to continue using these techniques to protect the genetic integrity of this endangered population until the natural run has increased sufficiently and hatchery supplementation is no longer required.

Develoument of new molecular markers will strengthen efforts to assess the effectiveness of winter-run supplementation. Different numbers and kinds of molecular markers are needed for different tasks. For example, assignment to winter run can be accomplished with an existing set of five to seven moderately polymorphic markers. However, determining parentage requires several highly polymorphic markers. and estimating average pairwise disequilibrium has an inherently high variance that can only be reduced by using as many markers as possible. Our ability to assign individuals to hatchery family origin using genetic techniques is dependent upon the type of molecular markers used. The more polymorphic the marker, the more likely different families will have unique alleles enabling them to be more easily distinguished from one another. Increasing the inventory of markers will reduce uncertainty in genetic assessments, increasing the positive feedback mechanism in the adaptive management framework.

Molecular markers will determine whether a naturally spawning population of winter run exists in Battle Creek. Due to year-round cold water springs originating from Mount Lassen, Battle Creek has the potential to support a naturally spawning winter-run population. By trapping and genotyping returning individuals, we can ascertain whether any non-hatchery individuals (identified by the absence of an adipose fin clip) are winter run and estimate the size of this population.

Assigning returning spawners to families created in the hatchery and improving run-size estimates of the natural population enables direct estimates of the effective population size of the hatchery release. Hedrick et al. (in review) have demonstrated that the predicted effective population sizes of the 1994 and 1995 hatchery releases are remarkably close to the direct estimates obtained from the returning spawners. Long-term estimates provide the opportunity to test the performance of a predictive model in an empirical context. These estimates are critical to the evaluation of the potential genetic impact of the artificial propagation program on the natural population (Hedrick et al. 1995, 2000). Long-term data are required to determine whether differential survival occurs among returning families. Because salmon return to spawn between two and four years of age, at least three years are required to adequately assess the return rate of a single cohort. In addition, year-to-year variation in environmental conditions (e.g., El Niño, La Niiia), which influence both the age and number of returning spawners, necessitate data collection on a longer time scale. Refining winter-run size estimates of the natural population by genotyping adult carcass returns and outmigrating juveniles will also lead to increased predictive power of the effective population size model.

Adaptive Management

This proposal can be considered both an assessment and a monitoring project linking annual and interannual data sets to management actions. Assessment and monitoring is created by examining genetic variation in temporal and spatial scales of each salmon run, since Central Valley stocks overlap in time and space. Documenting interannual variation provides insight into stock variation and responses to environmental change, either natural or anthropogenic. The positive feedback mechanisms that refocus practice at the Livingston Stone Hatchery and the Winter Run Captive Broodstock Program provide managers the ability to select broodstock harvest when winter and spring runs temporally overlap. Hybridization and significant loss of both winter and spring run resources (both listed species) will occur without this mechanism to refocus fish culture practice.

Educational Objectives

Although not educational in context, this project is linked with ongoing public education programs at BML and the California Academy of Sciences Steinhart Aquarium describing the Winter Run Captive Broodstock Program. This year a separate CALFED project submitted by the Sacramento River Discovery Center (SRDC) proposes *to* increase the educational awareness of the role of molecular biology in the conservation of Central Valley salmon resources. By increasing the number of venues and adding new multimedia materials, the SRDC project will reach an audience of over five million people. Its message will be to describe the value of maintaining biodiversity, the relationship of genetic variation to ecosystem function and the values of environmental services provided by healthy riparian habitat supporting salmon abundance in a sustainable fashion. The multimedia objectives include two videos: one targeting grades 5-6 to adult public education and the second aimed at agency personnel and decision makers. The latter aids in the adaptive management process by providing clear. concise explanations of how molecular and conservation biology operates in Central Valley stock assessment. creating feedback into the decision analysis framework guiding restoration process.

2. Proposed Scope of Work

Location of the Project

Genetic research and analysis in support of the USFWS's winter-run chinook captive propagationibroodstock program will take place at the Bodega Marine Laboratory (BML) in Bodega Bay, Sonoma County. Tissue samples from chinook salmon will be collected by USFWS personnel from monitoring and trapping locations along the upper Sacramento River in Shasta and Tehama Counties. Returning adults will be trapped at Keswick Dam (river mile 302), Red Bluff Diversion Dam (RBDD, river mile 243) and in Battle Creek (Coleman National Fish Hatchery). Outmigrating juveniles will be collected from rotary screw traps at RBDD and Battle Creek, and tissues from carcass surveys will be collected from the upper Sacramento River and Battle Creek. These locations are within the Sacramento River and North Sacramento Valley ecozones (Appendix **A:** 3.1, 3.2 and 4.4).

Approach

The USFWS conducts propagation and captive broodstock programs for endangered winter-run chinook salmon at the Livingston Stone National Fish Hatchery, located at the base of Shasta Dam on the Sacramento River. The program consists of collecting adult winter-run chinook from the mainstem Sacramento River, holding and spawning the adults, rearing the juveniles in the hatchery environment. then releasing them back into the mainstem Sacramento River. Initial broodstock selection for the propagation program is critical to the maintenance of genetic integrity of the winter-run population. At BML, research will entail characterization and identification of winter-run chinook salmon through molecular and population genetic techniques. Genetic analyses are made possible by the development of microsatellite DNA markers (i.e., loci). These markers have core DNA sequences of 2-4 nucleotides that are repeated multiple times at a particular site and are transmitted via both parents, thus providing a means of assessing family origin. Microsatellites are often highly variable within populations due to a high mutation rate. They are therefore more likely to reflect recent evolutionary events and have thus been applied in a wide variety of population genetic studies, especially for closely related populations (Jarne and Lagoda 1996). Microsatellites are amplified by the polymerase chain reaction (PCR) from small, non-lethal tissue samples (caudal fin clips) and rapidly typed using denaturing polyacrylamide gel electrophoresis and fluorescent imaging (Banks et al. 1999). The genotypes of individual fish are determined for five loci: Ots-2, *-3, -9, -10* (Banks et al. 1999) and *Oneμ13* (Scribner et al. 1996). If necessary, two additional markers are used (*Ots-104* and -107, Nelson and Beacham 1999). The odds of a given genotype being winter run are calculated as the ratio of genotypic frequencies in winter *vs.* other runs, using frequencies for the relevant spawning populations (Banks et al. 2000) and the computer program WHICHRUN developed at BML (Banks and Eichert 2000). This program differs from traditional mixed stock analysis techniques in that it makes run probability assessments for individual fish. For winter-run fish, the log of the odds score (LOD) is 2 or greater with the core five loci (the criterion agreed by the Genetics Subcommittee 2/27/98), or 1 or greater using a total of seven loci (as discussed by the Genetics Subcommittee 2/26/99).

Objective 1. Identify individual salmon adults for use in the USFWS's **winter-run chinook salmon captive propagation and broodstock programs.** All salmon returning to the main stem of the Sacramento River between February and July will be trapped at Keswick Dam and RBDD. numbered and fin clipped. The tissue will then be FedExed to BML overnight. Immediately upon arrival. DNA will be extracted from each tissue sample in triplicate (Chelex technique) and amplified (PCR) at the core five loci. Using WHICHRUN, each fish will be classed as winter or non-winter run based on its LOD score. If the LOD score is less than 2, but greater than zero, the fish will be genotyped at two additional loci and the LOD score again determined. We will re-evaluate the LOD score criterion for winter-run broodstock as new diagnostic loci are brought online. Computer simulations using genotypes in our extensive Central Valley chinook database (Banks et al. 2000) and the computer program WHICHLOCUS (developed at BML) will help to evaluate the likelihood that non-winter fish would be included and true winter fish excluded by any given LOD criterion.

Objective 2. Develop new molecular markers to determine the family origin of returning hatchery-bred fish. Assigning offspring of unknown parentage to family requires a suite of highly

polymorphic markers. While the core five dinucleotide loci currently used allow discrimination between winter and the other chinook runs, winter run is characterized by having markedly fewer alleles, thus making it more difficult to distinguish among winter-run families using these particular loci. However, the additional two tetranucleotide markers are more polymorphic in winter run and prove extremely useful in resolving pedigrees. Nonetheless, more polymorphic markers are required. We propose to optimize and test the Ots-200 series loci, recently developed at BML for identification of spring run, for use in winter-run pedigree analysis. We will continue to develop MHC (major histocompatibility complex) markers; we have characterized a class II gene (involved with recognition of bacterial and other extracellular antigens) and are in the process of examining a class I gene (involved with recognition of viral and other intracellular antigens). The class I gene is quite variable and should prove useful in family identification. In addition, in another project we will be using families of isogenic homozygous pink salmon to identify other genes in the MHC. We will then use the information from those screens to identify further MHC genes and their variants in winter run chinook. From this background, we will be able to determine the physical linkage relationship of these genes. We will use a computer program under development at BML (WHICHPARENT) to assign fish to family. This work is important not only in selecting non-related hatchery-bred individuals for incorporation in the captive broodstock program (of which 10% of the target capture rate is permitted), but also to confirm the effective population size model with direct estimates obtained from returning spawners. To this end. we will use this technique to make family assignments on fish collected as post-spawn winter carcasses from the main stem of the Sacramento River and Battle Creek, as well as those live trapped at RBDD and Keswick Dam.

Objective *3*. Genotype and identify to run origin (e.g., winter/non-winter) **salmon carcasses collected in mainstem Sacramento River or Battle Creek carcass surveys for** population **assessments and N_e validation.** Genetic analysis of tissue will be carried out on adult carcass samples to refine run-size estimates generated in adult carcass monitoring surveys in the mainstem Sacramento River and in Battle Creek. Since DNA from carcass samples is generally degraded compared to fresh tissue, we will use the Puregene DNA Isolation Kit (Gentra systems, Inc.) to obtain higher quality DNA and perform PCR for each individual in triplicate as a quality control measure. We will also explore alternative sources of DNA by extracting from scales and the operculum. Individuals will be genotyped at seven microsatellite markers and analysed for run identity using WHICHRUN. Any winter run fish of hatchery origin will be assigned to family using WHICHPARENT.

Objective 4. Genotype and identify to run origin juveniles collected from rotary screw trap operations at the Red Bluff Diversion Dam or in Battle Creek for population assessments and N_e validation. Genetic analysis of tissue will be carried out on juvenile samples to refine run-size estimates generated in juvenile monitoring surveys in the mainstem Sacramento River and in Battle Creek. Tissue from juveniles will be treated with Chelex as before and genotyped at seven loci. After analysing with WHICHRUN, the relatedness of winter-run juveniles will be determined. This will be achieved by the disequilibrium method (GENETIX v3.3, www.univ-montp.fr/~genetix/genetix.htm), although other methods, such as the computer program 'Relatedness' **(http://gsoft.smu.edu/GSoft.html)** and **a** program under development at **BML (SIBLINGS),** will be explored.

Objective 5. Genotype and identify to run origin returning spawners trapped in Battle Creek to determine whether a naturally spawning population of winter-run is sustained. Trapping in Battle Creek during the same time period that fish are trapped at Keswick Dam, and determining run origin using the same genetic techniques, will enable us to verify the existence of any naturally spawning winter-run population in Battle Creek.

Objective 6. Determine genetic impacts of the supplementation program on the naturally spawning population through genetic analysis, and verify/refine an effective population size (N_e) model. To analyze the effective population size of the winter run, we will estimate the effective population size for the fish released from the USFWS winter-run chinook salmon captive propagation/broodstock program using the model developed by Hedrick et al. (1995). This will then be verified by population genetic analysis using returning spawners, both by identifying them to family and by using changes in allele frequency over multiple generations to estimate effective population size. Other approaches to evaluate N_e, such as the linkage disequilibrium approach, will be tested.

Data Handling and Storage

The genetic results and analyses of the fish trapped at Keswick Dam will be faxed to USFWS at Red Bluff and Livingston Stone National Fish Hatchery usually within 24 hours, but no later than three working days, after receiving the tissue samples. Genetic data from live-trapped fish. carcasses and juveniles will be stored in a database (Paradox and/or Excel). Data generated by various computer programs and simulations will be stored as Excel or text files. Written and electronic copies of the results of the N_e analyses will be provided.

Expected Products/Outcomes

We will produce written quarterly progress reports and provide updates of research activities to the Genetics Subcommittee of the Winter-Run Chinook Captive Broodstock Committee. We will also prepare publications for peer-reviewed journals and give presentations (poster and/or oral) at scientific meetings.

Work Schedule

Task 1. February–July, years 1 and 2. Genotype all adult chinook salmon trapped at Keswick Dam and RBDD for potential use in the USFWS artificial propagation program ("Rapid response" genetic analysis). All samples to be analysed at 5 loci, or 7 loci if $0<LOD<2$.

Task 2. October 2001–September 2003. Develop additional polymorphic markers (e.g., Ots-200 series. MHC class 1 and 11) to aid in family origin and linkage disequilibrium analyses. Determine parentage of returning spawners of hatchery-origin to verify effective population size predictions.

Task 3. October–January, years 1 and 2. Genotype carcasses obtained in the Sacramento River and Battle Creek between April–August 2001 and 2002. Estimate the proportion of winter-run chinook. determine the parentage of hatchery-origin fish to verify N_e predictions, and assess temporal N_e variation. Test alternative DNA sources to fin tissue, such as scales and opercula.

Task 4. May–September, years 1 and 2. Genotype a subsample of outmigrating juveniles caught at RBDD and Battle Creek screw traps in July–December 2001 and 2002. Verify the proportion of winter-run chinook, verify non-relatedness, and estimate the N_e of the natural population through linkage disequilibrium analyses.

Task 5. February–July, years 1 and 2. Genotype all adult chinook salmon trapped in Battle Creek to determine any naturally-spawning winter run. All samples to be analyzed at 5 loci, or 7 loci if $0<LOD<2$.

Task *6*. October 2001–September 2003. Computer simulations using WHICHRUN and WHICHLOCI to test winterinon-winter assignment probabilities with additional loci. Refinement of computer program WHICHPARENT to aid in assigning returning hatchery-bred individuals to family. Development of computer program SIBLINGS to assess relatedness among juvenile samples.

Task 7. Arizona State University subcontract. October 2001–September 2003. Use genetic analyses from Tasks 2, 3 and 4 to verify the effective population size model and thus monitor potential genetic impacts of the artificial propagation program on the natural population.

Project Management. Ongoing. Provide status reports on completed work and research advances to CALFED, CVPIA, USFWS and present research updates at the Genetics subcommittee of the Winter-Run Chinook Captive Broodstock Committee. Prepare publications for refereed journals and presentations at scientific meetings.

Feasibility

The overriding goal of this proposal is to supplement the winter-run chinook population and provide an insurance policy against extinction. As this program is designed to supplement an endanpered population, attention to genetic considerations has remained a high priority and, since 1997. fundinp for genetic investigations has been sought and acquired through AFRP. Over the last three years. we have demonstrated that we can use molecular genetic resources developed at BML (Banks et al. 1999. 2000. Banks and Eichert 2000) to complete the work outlined in this proposal in a timely manner (see progress reports for 1998-99 to USFWS). We have also published our results on the impact of the supplementation program on the effective population size of the winter run in 1991-93 (Hedrick et al. 1995) and 1994-5 (Hedrick et al. 2000 and in review). BML maintains the ESA authorizations to receive and archive tissue samples under a CDFG MOU for California Endangered Species Act. Federal ESA compliance is maintained through separate ESA permits authorized by NOAA Fisheries.

Applicability to CALFED ERP Goals and Implementation Plan and CVPIA Priorities

ERP Goals and CVPIA Priorities

Winter-run chinook salmon are formally listed as endangered under the State and Federal Endangered Species Acts (ESAs). As such they are considered an "at-risk species" (Goal 1) and given highest priority for population restoration and legal recovery. As previously stated, genetic maintenance of this endangered population is critical to future recovery of this species. Collation of all information resulting from activities to maintain the genetic variation within this population will likely become the basis for the development of a Genetic Management Plan for winter-run chinook salmon. Information provided through the funding of this proposal will continue to provide information on the genetic integrity of the winter-run chinook salmon population. Additionally, the information will allow assessment of the effect of the supplementation program on the potential recovery of the species. Continued research: applied genetic analysis and general genetic guidance in this area is critical to the overall success of the program and the genetic resources of the species. The results of the genetic analysis increases the effectiveness of the restoration activity supported in this proposal through genetic validation of selected broodstock and evaluation of potential genetic impacts. Additionally, the proposed work continues to improve the scientific understanding of the endangered population of winter-run chinook salmon through identification and maintenance of the genetic integrity of the population, and assists in providing information useful in the estimation of population status (i.e., run-size).

Relationship to Other Ecosystem Restoration Projects

This project is inextricably linked to genetic research for the management and protection of endangered Central Valley chinook salmon currently funded by the Department of Water Resources. Specifically. the Central Valley project analyses samples of outmigrating juveniles from just below the spawning grounds, throughout the Delta, and towards the ocean to determine where and when winter run are present. Juveniles salvaged at the State and Federal water diversion facilities (Tracy, California) are also analyzed on a rapid response basis to establish winter run "take" at these facilities. The Central Valley work will give a better understanding of winter run outmigration behavior, which will in turn assist management and protection strategies. Given the close links between the two projects, considerable sharing of information and resources can take place.

Requests for Next-Phase Funding

This proposal represents the application of techniques devised and refined during three years of previous funding from AFRP through the USFWS. See the Appendix for a summary of the existing project.

Previous CALFED/CVPIA funding

1) Genetic Maintenance of Hatchery- and Natural-Origin Winter-Run Chinook Salmon (Cooperative agreement number 1448-11330-97-J194). Since 1998, BML has been subcontracted to carry out the genetic component of the endangered winter-run propagation program by USFWS-Red Bluff. These funds were acquired through AFRP (see the Appendix for a summary of the project to date).

2) Molecular Genetic Identification of Chinook Salmon Runs Focusing on Spring Run (D. Hedgecock, co-PI; Category III; Contract Number 97-0330-B-81182; administered through Department of Water Resources). The major accomplishments of this three year project (due to terminate on June 30, 2000), include creating a genomic DNA library for spring-run chinook salmon, cloning and screening microsatellites for spring-run discrimination, and developing and optimizing six of these markers for general use. Multiplexing the initial five core loci (*Ots-2*, -3, *-9*, *-10*, *Oneμ13*) was also achieved (Greig and Banks 1999). In addition, genetic analysis of an experimental ocean fishery to identify harvest of endangered chinook salmon and a preliminary characterization of Klamath Basin chinook salmon were completed with support from this project.

System-Wide Ecosystem Benefits

The benefits of the genetic techniques outlined in this proposal are multiple, since they can also be applied to populations other than the winter run. For example, by trapping and identifying (through genetic techniques) all salmon returning to the Keswick barrier dam, we can document not only the return of spawning winter-run chinook, but also other runs, specifically the endangered spring run. With the addition of more polymorphic markers which can discriminate among spring, fall and late-fall runs (all of which occur in the upper Sacramento River), our understanding of chinook salmon population genetics will be vastly improved. Similarly, genetic tools for population discrimination are highly relevant to Battle Creek restoration initiatives given that all four populations of chinook salmon occur in this watershed.

Qualifications

Dr. Dennis Hedgecock is responsible for project management, including supervising work in progress. preparation of periodic reports and publications for peer-reviewed journals, presentation of results at the Genetics Subcommittee meetings and at scientific meetings.

EDUCATION Ph.D. (Genetics), University of California, Davis, 1974
B.S. (Biology), St. Mary's College, California, (Magna cum Laude)

PROFESSIONAL EXPERIENCE

1990-present	Geneticist, Department of Animal Science, University of California, Davis and the Bodega Marine Laboratory (BML), Bodega Bay, California
1983-1990	Associate Geneticist, Department of Animal Science, UC Davis and BML
1978-1983	Assistant Geneticist, Department of Animal Science, UC Davis and BML
1974-1978	Postgraduate Research Geneticist, BML

SELECTED RELEVANT PUBLICATIONS

Hedgecock, D. 1994. Does variance in reproductive success limit effective population size of marine organisms? In ***Generics*** *and evolution of aquatic organisms.* A. Beaumont (Ed.), Chapman and Hall. London. pp. 122-134.

Pudovkin, A. I., D. V. Zaykin, and D. Hedgecock. 1996. On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Generics* 144:383-387.

Li, G., and D. Hedgecock. 1998. Genetic heterogeneity detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas* Thunberg), supports the hypothesis of large variance in reproductive success. *Can. J. Fish. Aquat Sci.* 55:1025-1033.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. **A.** Fitzgerald, S. M. Blankenship. and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus rshawyrscha).J. Hered.* 90:281-288.

Banks, M.A., V.K. Rashbrook, M.J. Calavetta, C.A. Dean and D. Hedgecock (2000). Microsatellite DNA variation in chinook salmon of California's Central Valley. *Can. J. Fish. Aquat Sci.* 57:1-14.

Vanessa Rashbrook M.S. (Staff Research Associate, 100%), has worked in the salmon genetics lab at BML for the past five years. Vanessa will continue to coordinate research, develop new markers, oversee the day-to-day activities of molecular characterization of samples and assist in preparing reports. publications and presentations. She will also order laboratory supplies and oversee budget matters.

Stephen Sabatino B.S. (Post Graduate Researcher, 100%), has 18 months experience at BML. Stephen will continue to assist in generating and analyzing data.

Will Eichert (Programmer III, 25%), will continue to develop computer programs and conduct computer simulations.

Dr. Philip Hedrick is a consultant on the project responsible for testing and refining models on effective population size.

EDUCATION

1960-1962	Hanover College, Indiana
1962-1963	American University of Beirut, Lebanon
1963-1964	B.A., Hanover College - Biology
1964-1966	M.S., University of Minnesota - Genetics
1966-1968	Ph.D., University of Minnesota - Genetics

RECENT PROFESSIONAL EMPLOYMENT

1988-1992	Professor, Pennsylvania State University
1998-present	Ullman Professor, Arizona State University

SELECTED RELEVANT PUBLICATIONS

Parker, K. M., R. J. Sheffer, and P. W. Hedrick. 1999. Molecular variation and evolutionarily significant units in the endangered Gila topminnow. *Cons. Biol.* 13:108-116.

Kim, T. J., K. M. Parker, and P. W. Hedrick. 1999. Major histocompatibility complex differentiation in Sacramento River Chinook salmon. *Generics* 151:1115-1122.

Hedrick, P. W. 1999. Perspective: highly variable genetic loci and their interpretation in evolution and conservation. *Evolution* 53:313-318.

Sheffer, R. J., P. W. Hedrick, and A. L. Velasco. 1999. Testing for inbreeding and outbreeding depression in the endangered Gila topminnow. *Anim. Cons.* 2:121-129.

Hedrick, P. W., T. E. Dowling, W. L. Minckley, B. D. DeMaris, and P. C. Marsh. 2000. Establishing a captive broodstock for an endangered species: Bonytail chub *(Gila elegans)* as a case study. *J. Hered* 91:35-39.

Hedrick, P.W., D. Hedgecock, S. Hamelberg, and S. J. Croci. 2000. The impact of supplementation in winter-run chinook salmon on effective population size. *J. Hered.* 91:112-116.

Hedrick, P.W. 2000. ***Genetics*** *of Populations*. Second Edition. Jones and Bartlett, Boston. pp. 553

Dan Garrigan (Ph.D. student, Arizona State University, 100%), will develop MHC markers.

Budget

Salaries: We request salaries for two full-time lab personnel (V. Rashbrook and S. Sabatino) to conduct DNA analysis on the several hundred samples anticipated for each task. We also require a part-time computer programmer (Will Eichert) to handle the increasing computer and statistical needs of this project. Dr. D. Hedgecock and V. Rashbrook will be responsible for project management. although Dr. Hedgecock is not requesting salary for his involvement. Estimated benefits constitute 20% (V. Rashbrook, W. Eichert) and 22% (S. Sabatino) of salaries, respectively.

	2001/2	2002/3
Vanessa Rashbrook, Staff Research Associate II (100%)	38,688	40.044
Stephen Sabatino, Post Graduate Researcher I (100%)	32,448	33.510
Will Eichert, Programmer III (25%)	13,512	13.987

Travel: We request support for travel to meetings of the Genetics Subcommittee of the \$'inter-Run Chinook Captive Broodstock Committee, and to scientific meetings relevant to the project. such as the Coastwide Salmonid Genetics Meeting.

Supplies: We will require reagents and materials used in molecular genetics labs, including expendables for PCR reactions (e.g., Taq polymerase enzyme, fluorescently labelled primers, tubes. lids and trays. pipette tips): chemical reagents for buffers, DNA extraction and polyacrylamide gels (e.g., Chelex. Purgene DNA Isolation Kit, acrylamide); glassware (e.g., gel plates); and computer supplies (e.g., software upgrades, zip discs and CDs for backup and storage, printer cartridges). Based on costs incurred in previous years, we anticipate requiring between $600 and $1000 per month per lab researcher for materials and supplies. We also request funds for phone and fax costs, publication costs and shipping by air courier (FedEx). The latter is required for transportation of tissue and/or DNA samples between BML and Livingston Stone National Fish Hatchery and between BML and other salmon genetics laboratories, including our Arizona State University subcontractor.

Equipment: We do not anticipate making any equipment purchases since our lab is fully equipped with the necessary PCR thermal cyclers, gel rigs, power supplies, laser scanners (FMBIO, Hitachi), and computers required to perform this research.

Service Contract: We require extended warranty on a thermal cycler (MJ Research). This machine performs PCR reactions essential to our research.

Overhead/Indirect Costs: Indirect cost rates are 10% for state resource agencies and 26% for federal. These rates are applied to the modified total direct costs, consisting of total direct costs excluding equipment items. Indirect cost covers routine laboratory maintenance, general office staff and administration costs, and local phone costs. In addition, for the first year only, indirect costs are charged on the first $25,000 of the Arizona State University subcontract.

Consultant/Subcontract: The amount charged by our Arizona State University subcontractor ($40,000) is devoted entirely to salaries, benefits and indirect costs ***(26%)*** by ASU.

Table 1. Annual and total budget assuming federal (26% overhead) or state (10%) funding source.

			Subject to Overhead										
Year	Task	Direct Labor Hours	Salary	Benefits	Travel	Supplies & Expendables	Service Contracts	ASU OH (26%)	Overhead (26%)	Total Cost (26%)	Overhead (10%)	Total Cost (10%)	
Year 1	Task 1: Rashbrook/Sabatino	870	$14,560	$3,074	$0	$2,750	$320	$0	$5,383	$26,087	$2,070	$22,774	
	Task 2: Rashbrook	348	$6,448	$1,290	$0	$2,749	$320	$0	$2,810	$13,617	$1,081	$11,888	
	Task 3: Rashbrook/Sabatino	870	$14,560	$3,074	$0	$2,750	$320	$0	$5,383	$26,087	$2,070	$22,774	
	Task 4: Rashbrook/Sabatino	870	$14,560	$3,074	$0	$2,750	$320	$0	$5,383	$26,087	$2,071	$22,775	
	Task 5: Rashbrook/Sabatino	870	$14,560	$3,075	$0	$2,750	$320	$0	$5,383	$26,088	$2,071	$22,776	
	Task 6: Eichert	522	$13,512	$2,702	$0	$0	$0	$0	$4,216	$20,430	$1,621	$17,835	
	ASU sub-contract*	2,213	$28,945	$2,801	$0	$0	$0	$8,254	$6,500	$46,500	$2,500	$42,500	
	Project Management	348	$6,448	$1,290	$1,500	$2,749	$0	$0	$3,117	$15,104	$1,199	$13,186	
Total Cost Year 1			$113,593	$20,380	$1,500	$16,498	$1,600	$8,254	$36,175	$200,000	$14,683	$176,508	
Year 2	Task 1: Rashbrook/Sabatino	870	$15,052	$3,328	$0	$2,882	$320	$0	$5,611	$27,193	$2,158	$23,740	
	Task 2: Rashbrook	348	$6,674	$1,402	$0	$2,881	$320	$0	$2,932	$14,209	$1,128	$12,404	
	Task 3: Rashbrook/Sabatino	870	$15,052	$3,328	$0	$2,882	$320	$0	$5,611	$27,193	$2,158	$23,740	
	Task 4: Rashbrook/Sabatino	870	$15,052	$3,328	$0	$2,882	$320	$0	$5,611	$27,194	$2,158	$23,740	
	Task 5: Rashbrook/Sabatino	870	$15,051	$3,328	$0	$2,881	$320	$0	$5,611	$27,192	$2,158	$23,738	
	Task 6: Eichert	522	$13,987	$2,937	$0	$0	$0	$0	$4,400	$21,324	$1,692	$18,616	
	ASU sub-contract*	2,213	$28,945	$2,801	$0	$0	$0	$8,254	$0	$40,000	$0	$40,000	
	Project Management	348	$6,674	$1,402	$1,500	$2,882	$0	$0	$3,239	$15,696	$1,245	$13,703	
Total Cost Year 2			$116,486	$21,854	$1,500	$17,290	$1,600	$8,254	$33,016	$200,000	$12,695	$179,682	
Total Project Cost			$230,079	$42,234	$3,000	$33,788	$3,200	$16,508	$71,191	$400,000	$27,381	$356,190	

*Note, Arizona State University (ASU) subcontractor is subject to UCD overhead only on first $25,000 in year 1; however, they have 26% OH rate of their own, which is part of the total sub-contract budget of $40,000 per year.

Compliance with Standard Terms and Conditions

The applicant will comply with the state and federal standard terms contained in Attachments D (State) and E (Federal).

Literature Cited

Banks, M.A., M.S. Blouin, B.A. Baldwin, V.K. Rashbrook, H.A. Fitzgerald, S.M. Blankenship and D. Hedgecock (1999). Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). *J. Hered.* 90: 281-288.

Banks, M.A. and W. Eichart (2000). WHICHRUN (version 3.2): a computer program for population assignment of individuals based on multilocus genotype data. *J. Hered.* 91: 87-89.

Banks, **M.A.,** V.K. Rashbrook, M.J. Calavetta, C.A. Dean and D. Hedgecock (2000). Microsatellite DNA variation in chinook salmon of California's Central Valley. *Can. J Fish. Aquat Sci.* 57: 1-14.

Bartley, D., G.A.E. Gall, B. Bentley, J. Brodziak, R. Gomulkiewicz and M. Mangel (1992). Geographic variation in population genetic structure of chinook salmon from California and Oregon. *Fish. Bull.* U.S.90:77-100.

Franklin, I.R. (1980). Evolutionary changes in small populations. In Soulé, M., ed. *Conservation Biology;* An *Evolutionary Ecological Perspective.* Sunderland, MA: Sinauer, pp. 135-149.

Greig, C. and M.A. Banks (1999). Five multiplexed micosatellite loci for rapid response run identification of California's endangered winter chinook salmon. *Anim. Gene?. 30:* 318-320.

Hedrick, P.W., D. Hedgecock, and S. Hamelberg (1995). Effective population size in winter-run chinook salmon. *Cons. Biol.* 9: 615-624.

Hedrick, P.W., D. Hedgecock, S. Hamelberg, and S.J. Croci (2000). The impact of supplementation in winter-run chinook salmon on effective population size. *J. **Hered.*** 9: 112-116.

Hedrick, P.W., V.K. Rashbrook, and D. Hedgecock (in review). Effective population size and N_e/N Ratio in returning winter-run chinook salmon. *Evolution.*

Lande, R. and G.F. Barrowclough (1987). Effective population size genetic variation and their use in population management. In Soulé, M.E., ed. *Viable Populations for Conservation.* New York. NY: Cambridge University, pp. 87-124.

Luikart, G. and J.M. Cornuet (1999). Estimating the effective number of breeders from heterozygote excess in progeny. *Genetics* 151: 1211-1216.

Nelson, R.J. and T.D. Beacham (1999). Isolation and cross species amplification of microsatellite loci useful for study of Pacific Salmon. *Anim. Genet.* 30 228-229.

Nunney, L. (1996). The influence of variation in female fecundity on effective population size. *Biol. J. Linn. Soc.* 59: 411-425.

Pudovkin, A.I., D.Y. Zaykin, and D. Hedgecock (1996). On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. *Genetics* 144: 383-387.

Ryman, N. and L. Laikre (1991). Effects of supportive breeding on the genetically effective population size. *Cons Biol. 5:* 325-329.

Scribner, K.T., J.R. Gust, and R.L. Fields (1996). Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. *Can. J. Fish. Aquat. Sci.* 53: 833-841.

Waples, R.S. (1991). Genetic methods for estimating the effective size of cetacean populations. In Hoelzel, A.R., ed., *Genetic Ecology of Whales and Dolphins.* (Intl. Whaling Comm., Special Issue 13), pp. 279-300.

Appendix: Summary **of** current project, **1997-2000**

Project Background. The winter-run chinook salmon propagation program (initiated in 1989). and the captive broodstock program (initiated in 1991) are recognized in the NMFS's draft Recovery Plan for this endangered species. In 1996, the USFWS initiated a two-year, self-imposed moratorium on the collection of naturally spawning adults from the Sacramento River. This was done in light of genetic integrity concerns of the captured adults. A separate stock discrimination project, supported with funding from the California Department of Water Resources, allowed our lab at BML to generate allele frequency data with microsatellite DNA for the four chinook salmon spawning stocks in the Sacramento River. The stock discrimination project, in conjunction with work funded through the winter-run chinook salmon captive broodstock program (1991-1997) and AFRP (1998-2000) resulted in the development of a set of DNA markers highly useful to discriminate winter-run chinook salmon from other runs of salmon (Banks et al. 2000). Additionally, AFRP funding supported investigations of the heritability and evidence of non-linkage of the most discriminatory loci (Banks et al. 1999). These investigations provide definitive support in justifying the discriminatory capability of the DNA marker set. The combination of genetic data generated by the winter-run chinook salmon captive propagation/brood stock programs and the stock discrimination project is now being used to confirm run-identity of the broodstock collected for the propagation program and to determine parentage of the returning spawners (Hedrick et al. in review).

Sacramento River and Battle Creek Trapping. In 1997 we handled a total of 116 fish in "rapid response" mode. Fish traps were placed in Battle Creek to capture individuals that had imprinted on Coleman National Fish Hatchery in the early days of the propagation program. Any winter-run fish (of hatchery origin) were relocated to the Sacramento River main stem. In 1998, when the moratorium on capture of naturally spawning winter run was lifted, individuals for the broodstock program were also trapped at Keswick Dam on the Sacramento River. In 1998 we genotyped a total of 268 fish from both sites. In 1999 we genotyped 112 fish, and this year to date (10 May 2000) we have analyzed 151 fish. Table 1 gives the numbers of fish caught at the two trapping sites and the numbers determined to be winter run, using molecular genetic techniques. Winter-run individuals caught at Keswick were transported to Livingston Stone National Fish Hatchery for artificial propagation.

Table 1. Summary of "rapid response" winter-run assessment, 1997-present.

YEAR	# FISH GENOTYPED		#WINTER RUN (#assigned to family)	
	Sacramento R.	Battle Cr.	Sacramento R.	Battle Cr.
1997	n/a	116	n/a	84 (72)
1998	152	117	9 (6)	11 (8)
1999	42	70	24 (-)	0 (-)
2000	98	53	59 (-)	5 (-)

Effective Population Size. Hedrick et al. (1995,2000) have applied the Ryman-Laikre model on effective population size to evaluate the genetic impact of a hatchery supplementation program for the endangered Sacramento River winter chinook salmon. In each year of this program, from 1991 through 1995, we have calculated N_{eh} from data on the number of progeny produced by each male and female brood fish. The N_e/N ratio for the naturally spawning population is assumed to have a lower bound of 0.1 (Bartley et al. 1992) and an upper bound of 0.33 (Waples, personal communication). Estimates of N_{eh}, x, and N_e with and without supplementation, for 1994 and 1995 are summarized in Table 2, in order to illustrate the impact of hatchery supplementation in years with different natural run sizes (189 *vs.* 1361, respectively). There are several important points to note. First, the supplementation program likely had little, or perhaps a slightly positive impact on winter-run effective population size in both years, *i.e,*. ranges for N_e were lower without than with supplementation (bottom line, Table 2). Second. x, the proportion of fish contributed by the hatchery, was high (0.41) in 1994, when run size was low. and low (0.08) in 1995, when run size was high. Estimates of x are based on numbers of females. their egg production, and the survival of these progeny from egg to smolt stages. For hatchery stocks. the egg to smolt survival is estimated to be 28.5%, about twice as high as estimates for egg to smolt survival in the wild, 14.7% (Hedrick et al. 1995). This boost in early survival is precisely what makes hatchery supplementation such an attractive idea. Third- ratios of effective to actual numbers of captive broodstock, N_{eh}/N_h, were 0.8 and 0.62 in 1994 and 1995, respectively, much higher than the N_e/N ratio for the naturally spawning population (0.1 to 0.33). Higher survival of hatchery offspring. coupled with higher N_e/N ratios and contributions that are inversely proportional to the wild stock size. can increase natural biodiversity. Hatchery enhancement does not necessarily constitute a threat to genetic resources: indeed, hatchery supplementation can help to retain biodiversity that would otherwise be lost from threatened and endangered populations.

Table 2. Summaries of estimates of Ryman-Laikre model parameters for the Sacramento River winter chinook for 1994 and 1995 (after Hedrick et al. 2000).

Parameter	1994	1995
Number of breeding parents ($N_f + N_m$)	26	42
N_{eh} (95% confidence interval)	23.2 (15.9, 30.8)	29.2 (21.3. 37.5)
N_{eh} / N_h ratio	0.8	0.62
Number of wild adults	189	1361
Number taken captive (N_h)	29	47
Difference	160	1314
Proportion from hatchery (x)	0.407	0.083
N_e, without supplementation	18.9 – 63.0	136.1 – 453.7
N_{ew} (lower and upper bounds)	16.0 – 53.3	131.4 – 438
N_e (lower and upper bounds)	34.3 – 72.8	150.7 – 463.6

UNIVERSITY OF CALIFORNIA, DAVIS

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BODEGA MARINE LABORATORY
P.O. BOX 247
BODEGA BAY, CALIFORNIA 91923

CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento, CA 95814

10 May. 2000

Re: Public Notification Requirement

To whom it may concern;

This letter is to confirm that the project proposal titled "Using Molecular Techniques to Preserve Genetic Integrity of Endangered Salmon in a Supplementation Program" constitutes research that will be conducted solely in the laboratory. We intend to carry out all proposed objectives at the Bodega Marine Laboratory, University of California – Davis. Given that none of the proposed work involves any physical action on the ground. such as restoration or construction, we are not subject to CALFED requirements to notify local governments of any intended land use.

Sincerely,

Dennis Hedgecock

Dennis Hedgecock
Geneticist

Environmental Compliance Checklist

All applicants must fill out this Environmental Compliance Checklist. Applications must contain answers to the following questions to be responsive and to be considered for funding. *Failure to answer these questions and include them with the application will result in the application being considered nonresponsive and not considered for funding.*

1. Do any of the actions included in the proposal require compliance with either the California Environmental Quality Act (CEQA), the National Environmental Policy Act (NEPA), **or** both?

   _____ YES ✓ NO

2. If you answered yes to # **1,** identify the lead governmental agency for CEQNNEPA compliance.

   _____
   Lead Agency

3. If **you** answered **no** to # 1, explain why CEQA/NEPA compliance is not required for the actions in the proposal.
   Proposal is entirely lab-based research.

4. If CEQA/NEPA compliance is required, describe how the project will comply with either or both of these laws. Describe where the project is in the compliance process and the expected date of completion.

5. Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?

   _____ **YES** ✓ **NO**

   If yes, the applicant must attach written permission for access from the relevant properly owner(s). Failure to include written permission for access may result in disqualification of the proposal during the review process. Research and monitoring field projects for which specific field locations have not been identified will be required to provide access **needs** and permission for access with ***30*** days of notification of approval.

6. **Please indicate what permits or other approvals may be required for the activities contained in your proposal. Check all boxes that apply.**

LOCAL		
Conditional use permit	—	
Variance	—	
Subdivision Map **Act** approval	—	
Grading permit	—	
General plan amendment	—	
Specific plan approval	—	
Rezone		—
Williamson Act Contract cancellation	—	
Other _____ (please specify)		
None required	✓	
STATE		
CESA Compliance	✓	(CDFG)
Streambed alteration permit	—	(CDFG)
CWA § **401** certification	—	(RWQCB)
Coastal development permit	—	(Coastal Commission/BCDC)
Reclamation Board approval	—	
Notification	—	(DPC, BCDC)
Other _____ (please specify)		
None required	—	
FEDERAL		
ESA Consultation	✓	(USFWS)
Rivers & Harbors Act permit	—	(ACOE)
CWA § **404** permit	—	(ACOE)
Other _____ (please specify)		
None required	—	

DPC = Delta Protection Commission
CWA = Clean Water Act
CESA = California Endangered Species Act
USFWS = U.S. Fish and Wildlife Service
ACOE = U.S. Army Corps of Engineers

ESA = Endangered Species **Act**
CDFG = California Department of Fish and Game
RWQCB = Regional Water Quality Control Board
BCDC= Bay Conservation and Development Comm.

Land Use Checklist

All applicants must fill out this Land Use Checklist for their proposal. Applications must contain answers to the following questions to be responsive and to be considered for funding ***Failure to answer these questions and include them with the application will result in the application being considered nonresponsive and not considered for funding.***

1. Do the actions in the proposal involve physical changes to the land (i.e. grading, planting vegetation, or breeching levees) or restrictions in land use (i.e. conservation easement or placement of land in a wildlife refuge)?

   _____ YES ✓ **NO**

2. If **NO** to # 1, explain what type of actions are involved in the proposal (i.e., research only, planning only).
   Lab research only.

3. **If** YES to # **1,** what is the proposed land use change or restriction under the proposal?

4. **If** YES to # **1,** is the land currently under a Williamson Act contract?

   _____ YES _____ **NO**

5. **If** YES to # **1,** answer the following:

   Current land use _____
   Current zoning _____
   Current general plan designation _____

6. *If* YES *to #1*, is the land classified as Prime Farmland, Farmland **of** Statewide Importance or Unique Farmland **on** the Department **of** Conservation Important Farmland Maps?

   _____ YES _____ **NO** _____ **DON'T KNOW**

7. **If** YES to # **1,** how many acres **of** land will be subject to physical change or land use restrictions under the proposal?
   _____

8. If YES to # **1,** is the property currently being commercially farmed or grazed?

   _____ YES _____ **NO**

9. **If** YES to **#8,** what are the number of employees/acre _____
   the total number of employees _____

10. **Will** the applicant acquire any interest in land under the proposal (fee title or a conservation easement)?

_____ **YES** ✓ **NO**

11. What entity/organization will hold the interest?_____

12. **If YES** to # **10,** answer the following:

Total number of acres to be acquired under proposal _____
Number of acres to be acquired in fee _____
Number of acres to be subject to conservation easement _____

13. For all proposals involving physical changes to the land or restriction in land **use,** describe what entity or organization will:

manage the property _____

provide operations and maintenance services _____

conduct monitoring _____

14. For land acquisitions (fee title or easements), will existing water rights also be acquired?

_____ **YES** _____ NO

15. **Does** the applicant propose any modifications to the water right *or* change in the delivery ***of*** the ***water?***

_____ **YES** ✓ **NO**

16. **If YES** to # **15,** describe_____

APPLICATION FOR FEDERAL ASSISTANCE

OMB Approval No. 0348-0043

2. DATE SUBMITTED	Applicant Identifier
5/10/00	
3. DATE RECEIVED BY STATE	State Application Identifier
4. DATE RECEIVED BY FEDERAL AGENCY	Federal Identifier

1. TYPE OF SUBMISSION

Application ☐ Construction ☐ Non-Construction

Preapplication ☐ Construction ☐ Non-Construction

5. APPLICANT INFORMATION

Legal Name: **Regents of the University of California**

Organizational Unit: **Bodega Marine Laboratory**

Address *(give city, county,* State, *and zip code):* **Office of the Vice Chancellor for Research**
410 **Mrak Hall, One Shields Avenue**
Davis CA *95616*

Name and telephone number of person to be contacted on matters involving this application *(give* area *code)*

Rene Domino *530-752-3764* **rhdomino@ucdavis.edu**

6. EMPLOYER IDENTIFICATION NUMBER *(EIN):* 94-6036494

7. TYPE OF APPLICANT: *(enter appropriate letter in box)* I

A. State
B. County
C. Municipal
D. Township
E. Interstate
F. Intermunicipal
G. Special District
H. Independent School Dist.
I. State Controlled Institution of Higher Learning
J. Private University
K. Indian Tribe
L. Individual
M. Profit Organization
N. Other (Specify) _____

8. TYPE OF APPLICATION

☐ New ☐ Continuation ☐ Revision

If Revision, enter appropriate letter(s) in box(es) ☐ ☐

A. Increase Award B. Decrease Award C. Increase Duration
D. Decrease Duration Other *(specify):*

9. NAME OF FEDERAL AGENCY:

CALFED

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER ☐☐-☐☐☐

TITLE

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Using Molecular Techniques to Preserve Genetic Integrity of Endangered Salmon in a Supplementation Program

12. AREAS AFFECTED BY PROJECT *(Cities, Counties, States, etc.):*

Sonoma County

13. PROPOSED PROJECT		14. CONGRESSIONAL DISTRICTS OF	
Start Date	Ending Date	a. Applicant	b. Project
10/01/01	09/30/03	III	VI

15. ESTIMATED FUNDING:

a. Federal	$ 400,000 00
b. Applicant	$ 00
c. State	$ 00
d. Local	$ 00
e. Other	$ 00
f. Program Income	$ 00
g. TOTAL	$ 400,000 00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE _____

b. No. ☐ PROGRAM IS NOT COVERED BY E. 0.12372
☐ OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?

☐ Yes If "*Yes,*" attach an explanation. ☐ No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT, THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED.

a. Type Name of Authorized Representative	b. Title	c. Telephone Number
	Sandra M. Dowdy Contracts and Grants Analyst	(530) 752-2075
d. Signature of Authorized Representative		e. Date Signed
Sandra M. Dowdy		MAY 12 2000

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Prescribed by OMB Circular A-102

BUDGET INFORMATION - Non-Construction Programs

OMB Approval No. 0348-0044

SECTION A - BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. -$400,000		$	$	$	$	$ $400.000
2.						
3.						
4.						
5. Totals $400,000		$	$	$	$	$

SECTION B - BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY (1)	(2)	(3)	(4)	Total (5)
a. Personnel	$ 84,648	$ 87,541	$	$	$ 172,189
b. Fringe Benefits	17,579	19,053			36,632
c. Travel	1,500	1,500			3,000
d. Equipment					
e. Supplies	16,498	17,290			33,788
f. Contractual	40,000	40,000			80,000
g. Construction					
h. Other	1,600	1,600			3,200
i. Total Direct Charges (sum of 6a-6h)	161,825	166,984			328,809
j. Indirect Charges	38,175	33,016			71,191
k. TOTALS (sum of 6i and 6j)	$ 200,000	$ 200,000	$	$	$ 400,000
7. Program Income	$	$	$	$	$

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Prescribed by OMB Circular A-102

Previous Edition Usable

SECTION C - NON-FEDERAL RESOURCES

(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e) TOTALS
8.	$	$	$	$
9.				
10.				
11.				
12. TOTAL *(sum of lines 8-11)*	$	$	$	$

SECTION D - FORECASTED CASH NEEDS

	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
13. Federal	$ 200,000	$ 50,000	$ 50,000	50,000	$ 50,000
14. Non-Federal					
15. TOTAL *(sum of lines 13 and 14)*	$	$	$	$	$

SECTION E - BUDGET ESTIMATES OF FEDERAL FUNDS NEEDED FOR BALANCE OF THE PROJECT

(a) Grant Program	FUTURE FUNDING PERIODS (Years)			
	(b) First	(c) Second	(d) Third	(e) Fourth
16. 200,000	$ 50,000	$ 50,000	$ 50,000	$ 50,000
17.				
18.				
19.				
20. TOTAL *(sum of lines 16-19)*	$	$	$	$

SECTION F - OTHER BUDGET INFORMATION

21. Direct Charges: $328,809

22. Indirect Charges: 71,191 (26%)

23. Remarks:

OMB Approval No. 0348-0040

ASSURANCES - NON-CONSTRUCTION PROGRAMS

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

NOTE: Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant, I certify that the applicant:

1. Has the legal authority to apply for Federal assistance and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management and completion of the project described in this application.

2. Will give the awarding agency, the Comptroller General of the United States and, if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.

3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.

4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.

5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).

6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. §§1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. §794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. §§6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) §§523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. §§290 dd-3 and 290 ee 3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. §§3601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and, (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.

7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally-assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.

8. Will comply, as applicable, with provisions of the Hatch Act (5 U.S.C. §§1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§276a to 276a-7), the Copeland Act (40 U.S.C. §276c and 18 U.S.C. §874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§327-333), regarding labor standards for federally-assisted construction subagreements.

10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is $10,000 or more.

11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§1451 et seq.); (f) conformity of Federal actions to State (Clean Air) Implementation Plans under Section 176(c) of the Clean Air Act of 1955, as amended (42 U.S.C. §§7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended (P.L. 93-523); and, (h) protection of endangered species under the Endangered Species Act of 1973, as amended (P.L. 93-205).

12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.

13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. §470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. §§469a-1 et seq.).

14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.

15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. §§2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.

16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§4801 et seq.) which prohibits the use of lead-based paint in construction or rehabilitation of residence structures.

17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, "Audits of States, Local Governments, and Non-Profit Organizations."

18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations, and policies governing this program.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
Sandra M. Dowdy	Sandra M. Dowdy Contracts and Grants Analyst
APPLICANT ORGANIZATION	DATE SUBMITTED
THE REGENTS OF THE UNIVERSITY OF CALIFORNIA	MAY 12 2008

Standard Form 4248 (Rev. 7-97) Back

U.S. Department **of** *the* Interior

Certifications Regarding Debarment, Suspension and Other Responsibility Matters, Drug-Free Workplace Requirements and Lobbying

Persons signing this form should refer to the regulations referenced below for complete instructions:

Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions - The prospective primary participant further agrees by submitting this proposal that it will include the clause titled, "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions. See below for language to be used; use this form for certification and sign; or use Department of the Interior Form **1954 (DI-1954).** (See Appendix A of Subpart D of **43** CFR Part **12.)**

Certification Regarding Debarment, Suspension, Ineligibility and Voluntaiy Exclusion - Lower Tier Covered Transactions - (See Appendix B of Subpart D of **43** CFR Part 12.)

Certification Regarding Drug-Free Workplace Requirements - Alternate I. (Grantees Other Than Individuals) and Alternate II. (Grantees Who are Individuals) - (See Appendix C of Subpart D of **43** CFR Part **12.)**

Signature on this form provides for compliance with certification requirements under **43** CFR Parts **12** and **18.** The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of the Interior determines to award the covered transaction, grant, cooperative agreement or loan.

PARTA: Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions

CHECK __ *IF THIS CERTIFICATION* IS FOR A PRIMARY COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency:

(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

(2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PARTB: Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions

CHECK __ *IF THIS CERTIFICATION IS FOR A* LOWER *TIER* COVERED TRANSACTION AND *IS* APPLICABLE,

(1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

DI-2010
March **1995**
(This form **consolidates** DI-1953, DI-1954, DI-1955. DI-1956 and DI-1963)

PARTC: Certification Regarding Drug-Free Workplace Requirements

CHECK __ IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS NOT AN INDIVIDUAL.

Alternate I. (Grantees Other Than Individuals)

A. The grantee certifies that it will or continue to provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing an ongoing drug-free awareness program to inform employees about--
(1) The dangers of drug abuse in the workplace;
(2) The grantee's policy of maintaining a drug-free workplace;
(3) Any available drug counseling, rehabilitation, and employee assistance programs; and
(4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will --
(I) Abide by the terms of the statement; and
(2) Notify the employer in writing of tis or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;

(e) Notifying the agency in writing, within ten calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to every *gal* officer on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification number(s) of each affected grant;

(f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted --
(I) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or
(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b), (c), (d), (e) and (f).

B. The grantee may insert in the space provided below the site(s) for the performance of work done in connection with the specific grant:

Place of Performance (Street address, city, county, state, zip code)

_____

_____

_____

Check __ if there are workplaces on file that are not identified here

PART D: Certification Regarding Drug-Free Workplace Requirements

CHECK — IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS ANINDIVIDUAL.

Alternate II. (Grantees Who Are Individuals)

(a) The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant;

(b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction, in writing, within 10 calendardavs *of* the conviction. to the grant officer or other designee, unless the Federal agency designates a central point for the receipi of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant.

DI-2010
March 1995
(This form consolidates DI-1953, DI-1954
DI-1955. DI-1956 **and** DI-1963)

PARTE: Certification Regarding Lobbying
Certification for Contracts, Grants, Loans, and Cooperative Agreements

CHECK __ IF *CERTIFICATION* IS FOR THE AWARD OF ANY *OF* THE *FOLLOWING* AND *THE* AMOUNT EXCEEDS *$100,000:* A FEDERAL GRANT OR COOPERATIVE AGREEMENT, *SUBCONTRACT,* OR *SUBGRANT* UNDER THE GRANT OR COOPERATIVE AGREEMENT.

CHECK __ IF *CERTIFICATION* IS FOR THE AWARD OF A FEDERAL LOAN EXCEEDING THE AMOUNT *OF $150,000,* OR A *SUBGRANT* OR *SUBCONTRACT EXCEEDING $100,000,* UNDER *THE* LOAN.

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, and officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying." in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31, U.S. **Code.** Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

As the authorized certifying official, I hereby certify that the above specified certifications are true

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL Sandra M. Dowdy

TYPED NAME AND TITLE Sandra M. Dowdy
Contracts and Grants Analyst

DATE MAY 12 2008

DI-2010

March 1995

(This form consolidates DI-1953, DI-1954, DI-1955. DI-1956 **and** DI-1963)

United States Department of the Interior

FISH AND WILDLIFE SERVICE
NORTHERN CENTRAL VALLEY FISH AND WILDLIFE OFFICE
10950 Tyler Road
Red Bluff, CA 96080

10 May 2000

CALFED Bay-Delta Program Office
1416 Ninth Street, Suite 1155
Sacramento, California 95814

Subject: **Letter of Support for a Research Proposal Entitled:**
Using molecular techniques to preserve genetic integrity of endangered salmon in a supplementation program
Submitted by U.C. Davis' Bodega Marine Laboratory

The **U.S.** Fish and Wildlife Service's Northern Central Valley Fish and Wildlife Office formally submits this letter to CALFED in support of the genetics research proposed by U.C. Davis' Bodega Marine Laboratory. The genetic investigations as described in the proposal remain an integral part of the U.S. Fish and Wildlife Service's monitoring and propagation programs for endangered winter-run chinook salmon. Further, through the development and implementation of molecular genetic techniques to preserve the genetic integrity of endangered winter-run chinook salmon, the work supports the CALFED goal of restoration and recovery of this "at-risk species."

The objectives as presented in the proposal are fully related to a number of other actions/activities. As described in the proposal, tissue samples collected in a number of ongoing and proposed **U.S.** Fish and Wildlife Service field programs will be submitted to the Bodega Marine Laboratory to undergo genetic analyses. The results of these analyses support the actions/activities designed to develop winter-run chinook salmon population abundance estimates (i.e., winter-run chinook salmon mainstem carcass surveys and mainstem juvenile monitoring), and/or directly assist in the recovery of this species (i.e., winter-run chinook salmon supplementation program and Battle Creek restoration). Much of the work as described in the proposal has received previous funding from AFRP and remains in direct support of CALFED and CVPIA/AFRP goals and objectives.

Sincerely,

Scott Hamelberg
Assistant Project leader

ASU
ARIZONA STATE UNIVERSITY

May 5,2000

LaRee Maguire
University of California – Davis
Bodega Marine Lab
2099 Westside Road
Bodega Bay, CA 94923

Subject: ASU Proposal No. 00-1367

Enclosed is the original of an application for support entitled "Using Molecular Techniques to Preserve Genetic Integrity of Endangered Salmon in a Supplementation Program." The University's principal investigator for this work is Professor Phil Hedric of the Department of Biology. Any resulting award should reflect the recipient as, "Arizona Board of Regents for and on behalf of Arizona State University."

Your consideration of this application is appreciated. Please contact Professor Hedric at (480) 965-0799 if you have questions regarding the technical portions or Joseph Wessels at (480) 965-1427 if you have any administrative, budgetary or award questions.

Gary Delago
Interim Director

lb

Enclosures

MAIN CAMPUS
OFFICE OF RESEARCH & CREATIVE ACTIVITIES
PO BOX 871603. TEMPE, AZ 85287-1603

ASU

ARIZONA STATE UNIVERSITY

May 3, 2000

To whom it may concern;

I agree to carry out the collaborative work outlined in the project "Using Molecular Techniques to Preserve Genetic Integrity of Endangered Salmon in a Supplementation Program" as proposed in the Cal Fed proposal.

Yours sincerely,

Phil Hedrick
Ullman Professor

MAIN CAMPUS
COLLEGE OF LIBERAL ARTS AND SCIENCES
Department of Biology
PO BOX 871501. TEMPE, AZ 85287.1501
(602) 365-3571 FAX: (602) 965.2519

SCOPE OF WORK
(Subcontract to Arizona State University)

(1) Estimate the effective population size for the fish released from the USFWS winter-run Chinook salmon captive propagation/broodstock program using the model developed by Philip Hedrick.

(2) Verify the effective population size model developed by Philip Hedrick using population genetic analysis on the returning spawners.

(3) Assist BML staff on matters related to population genetic analysis of winter-run chinook salmon.

(4) Develop further approaches to estimate the effective population size of the natural run of winter-run chinook salmon.

(5) We will continue *to* develop MHC (major histocompatibility complex) markers for use in identification of winter run chinook salmon. We have characterized a class II (genes involved with recognition of bacterial and other extracellular antigens) gene that is now being used and are in the process of examining a class I (genes involved with recognition of viral and other intracellular antigens) gene, The class I gene is quite variable and should prove useful in identification. In addition, in another project we will be using families of isogenic homozygous pink salmon to identify other genes in the MHC. We will then use the information from those screens to identify further MHC genes and their variants in winter run chinook. From this background, we will be able to determine the physical linkage relationship of these genes.

(6) Provide updates of these research activities to the Winter-Run chinook Salmon Captive Broodstock Committee and other subcommittees and prepare publications for peer-reviewed journals.

INSTITUTIONAL ENDORSEMENT

Gary Delago, Interim Director
OFFICE OF RESEARCH & CREATIVE
ARIZONA STATE

Budget (2001-2002)

Salary		
Philip Hedrick (Ullman Professor)	0.72 months	8,785
Dan Garrigan (Ph.D. student)	12.0 months	20,160
Benefits		
Philip Hedrick (25%)		2,196
Dan Garrigan (3%)		605
Subtotal		31,746
Indirect Costs (26%)		8.254
Total Year I		$40,000

Budget (2002-2003)

Salary		
Philip Hedrick (Ullman Professor)	0.72 months	8,785
Dan Garrigan (Ph.D. student)	12.0 months	20,160
Benefits		
Philip Hedrick (25%)		2,196
Dan Garrigan (3%)		605
Subtotal		31,746
Indirect Costs (26%)		8,254
Total Year II		$40,000
Cumulative Total (2001-2003)		$80,000